REMARKS

I. Support for the Amendments to the Claims

Claims 1-13, 29-31, and 54-64 are currently in the application. Claim 1 has been amended and new claims 29-53 have been added to the present application. Claims 14-28 were previously withdrawn Claims 1-2 and 30 have been amended, previously withdrawn claims 42-53 have been cancelled, and new claims 54-64 have been added to the present application. Previously withdrawn claims 42-53 have been cancelled without prejudice to their pursuit in an appropriate divisional or continuation application. Claims 14-28 and 32-41 are withdrawn.

Support for the amendments to claims 1-2 and 30 and for new claims 54-64 can be found in the original specification, Examples, Figures, and claims. Additional support for the amendments to claim 1 and 30 and for new claims 29-53 can be found in the language of original claims 1-13 and from page 3, line 19, to page 4, line 6; on page 5, lines 5-13; from page 5, line 23, to page 10, line 11; in the Examples; and in the Figures. Additional support for the amendments to claims 1 and 30 and for new claims 54-64 can be found, e.g., from page 5, line 23, to page 8, line 6; from page 8, line 27, to page 9, line 25; in Example 1; and in the Figures, especially Figures 3-5. Additional support for the amendment to claim 2 can be found in original claim 2. Claim 2 has been amended merely to make a grammatical correction (the inadvertently omitted "or"). Additional support for new claim 54 can be found in original claims 1, 5, 7-10, and 12. Additional support for new claim 55 can be found in original claims 1-2. Additional support for new claim 56 can be found in original claims 1 and 3. Additional support for new claim 57 can be found in original claims 1 and 4. Additional support for new claim 58 can be found in original claims 1 and 5-6. Additional support for new claim 59 can be found in original claims 1 and 10-11.

II. Status of the Claims

Claims 1-28 were previously in the application. The claims were subject to an Election/Restriction Requirement, and claims 1-13 (Group I) were elected with traverse.

Claims 1-13, 29-31, and 54-64 are currently in the application. Claim 1 has been amended and new claims 29-53 have been added to the present application. Claims 14-28 were previously withdrawn Claims 1-2 and 30 have been amended, previously withdrawn claims 42-53 have been cancelled, and new claims 54-64 have been added to the present application. Previously withdrawn claims 42-53 have been cancelled without prejudice to their pursuit in an appropriate divisional or continuation application. Claims 14-28 and 32-41 are withdrawn.

III. The Priority Claim

The present application is a 35 U.S.C. §371 national stage of PCT application PCT/US2004/021637, filed July 6, 2004, which claims the priority benefit of U.S. Provisional Application Serial No. 60/485,509, filed July 6, 2003, and U.S. Provisional Application Serial No. 60/485,607, filed July 7, 2003, the disclosures of all of which are incorporated herein by reference. **Applicants respectfully request acknowledgement of the priority claim.**

IV. The Drawings

Applicants respectfully request consideration and acceptance of the drawings.

V. The Information Disclosure Statements

Applicants thank the Examiner for acknowledging the Information Disclosure Statements of September 24, 2008, and December 16, 2008.

VI. The Restriction Requirement

Applicants respectfully request the Examiner to consider whether any rejoinder of claims (e.g., Group IV [claim 25]) is possible at this stage in the prosecution should the remaining claims prove allowable. In particular, Applicants respectfully request the Examiner to re-consider the restriction requirement with respect to claims 32-41. Applicants note that any search or examination of these claims would overlap significantly with the search or examination of claims 1-3, 5, and 10. If discussion of any amendment or remark made herein would advance this important case to allowance, the Examiner is invited to call Applicants' undersigned representative as soon as convenient.

VII. The Rejection of Claim 30 under 35 U.S.C. §112, Second Paragraph, is Traversed, but Accommodated

The Examiner has rejected claim 30 under 35 U.S.C. §112, second paragraph, for allegedly failing to particularly point out and distinctly claim the subject matter. Applicants respectfully disagree, but have amended claim 30 to further prosecution in a timely manner.

The Patent Office alleges that claim 30 is indefinite with respect to "a reversible differentiation hotspot," because it is "uncertain into what cell type the stem cells would de-differentiate or reverse." Applicants respectfully submit that cellular de-differentiation is a concept known and understood for many years by those of ordinary skill in the art.

Applicants respectfully submit that claim 30 fulfills the requirements of 35 U.S.C. §112, second paragrph, thereby placing this claim in condition for allowance, and request the Examiner's reconsideration accordingly.

VIII. The Rejection of Claims 1-4, 7-9, 12-13, and 29-31 Under 35 U.S.C. §102(b) over Hagihara is Traversed

The Examiner has rejected claims 1-4, 7-9, 12-13, and 29-31 under 35 U.S.C. §102 for alleged anticipation by Hagihara et al. (J. Immunol. Methods 253: 45-55 [2001]). Applicants traverse the rejection and respectfully request reconsideration of these claims.

The Patent Office alleges in pertinent part:

> Hagihara et al. disclose a method for the production of differentiated hematopoietic cells including dendritic cells wherein the method comprises 1) step of culturing CD34+ bone marrow stem cells "under conditions that promote synchronous progress through the cell" that are contacting and culturing the cells with a medium comprising steel factor, thrombopoietin and FLT-3 ligand; 2) subsequent step of contacting the cells with growth factor GM-CSF at a "predetermined"phase or time of cell cycle; and 3) subculturing the cells with a growth factor GM-CSF for up to 14 days or about 14 days. (entire document including abstract and page 49 at section 2.4 "Culture system"). The method taught by Hagihara et al. comprises identical active steps and it results in the production of the differentiated hematopoietic cells as required by the claimed method and, thus, the cited reference by Hagihara et al. clearly anticipates claimed invention of the instant claims 1-4 and 13. Although production or generation of dendritic cells is a primary goal of the cited reference by Hagihara et al., the dendritic cells were not the sole cellular product of the disclosed culturing method and, thus, the final subculture after subculturing with maturation factors including GM-CSF and/or steel factor is reasonably expected to "comprise" at least some amounts of megakaryocytes, granulocytes and platelets within the broadest reasonable meaning of the claims 7-9 and 12.
>
> With respect to newly added claims 29-31 it is noted that the cited method of hagihara et al results in the production of differentiated hematopoietic cells, and, thus, the step (b) of contacting the cells with a factor inherently takes place at the moment of "hotspot" that "favors" differentiation or specific differentiation within generic mean of the claims. [Pp. 4-5.]

Applicants respectfully disagree.

For example, elsewhere in the Office Action, the Patent Office actually concedes:

> The cited reference by **Hagihara** et al **does *not* clearly recognize that the cell culturing in the presence of steel factor (SCF), thrombopoietin (TPO) and FLT-3 ligand (FLT3) promotes synchronous progression of cells through the cell cycle**. [P. 6, all emphasis added; see also p. 9.]

In its rebuttal (Office Action, p. 8), the Patent Office alleges that the above argument is Applicants' argument when in fact it is the Patent Office's own argument, both in the previous Office Action and in the present Office Action, as quoted above. This statement is *prima facie* incompatible with an allegation of anticipation, particularly with respect to the limitations as recited in claim 3:

> 3 (previously presented). The method of claim 1, wherein culturing the cells under **conditions that promote synchronous progression through the cell cycle** comprises **culturing the cells in the presence of steel factor, thrombopoietin, and FLT3-ligand**.

As noted previously, not only does Hagihara fail to disclose synchronous progression of cells through the cell cycle, Hagihara also does not disclose contacting synchronously cycling cells with a growth factor or cytokine "at a predetermined phase of the cell cycle."

Instead, the cells in Hagihara were subsequently subcultured "every week" with no disclosure regarding the timing of the subculturing with regard to the cell cycle. Hagihara does not even disclose whether the subculturing of the cells took place at the same time of day on the same day of "every week." (Applicants also wish to note respectfully that CD34+ cells are not necessarily stem cells.)

In its rebuttal (Office Action, p. 8), the Patent Office alleges:

....Applicants further argue (page 15, par. 1) that the term "every week" is not the time for "predetermined phase of the cycle". Yet, the claims are not limited by time. [P. 8.]

Applicants agree that the claims are not limited by time, which forms the very crux of this point of Applicants' argument. As Applicants previously argued, and currently maintain, the claims are limited by the phase of the cell cycle, which Hagihara is not. Rather, Hagihara discloses a vague, imprecise, and random period of time ("every week" – without even any discussion of the day, time, or duration of cell cycle), unlike the Applicants, whose claims are directed to a specific phase of the cell cycle ("predetermined phase of the cycle"), regardless of when in time that phase occurs. In essence, Applicants are claiming a method based on cell cycle, while Hagihara discloses a method based on time. Even if it were possible to know the duration of the cell cycle in numbers of hours (and it is not), Hagihara's vague, imprecise, and random "every week" neither discloses nor suggests a consistently specific cell cycle phase.

Applicants respectfully submit, therefore, that it cannot be concluded by one of ordinary skill in the art that Hagihara subcultured synchronously cycled cell cultures with a growth factor or cytokine "at a predetermined phase of the cell cycle."

Moreover, the Examiner's attention is respectfully drawn to the Declaration of Dr. Peter Quesenberry, who is presently a Professor of Medicine at the Warren Alpert Medical School of Brown University and Rhode Island Hospital in Providence, RI (since 2006); a Professor of Medicine at Boston University in Boston, MA (since January 2001); Director of the Division of Hematology/Oncology at Brown Medical School and Lifespan Medical Center at Rhode Island Hospital and Miriam Hospital in Providence, RI (since October 2006); the Paul Calbresi, MD, Professor in Oncology at Brown University and Rhode Island Hospital in Providence, RI (since October 2006); a member of the Board of Trustees of Roger Williams Medical Center in Providence, RI (since 2004); and a member of the National Board of Trustees for the Leukemia Society of America (since 1984).

As noted in Dr. Quesenberry's Declaration, Hagihara neither describes nor suggests the selection of a desired cell cycle specific hematopoietic cell type to be differentiated from the synchronous purified bone marrow stem cells at a phase of the cell cycle favoring a specific differentiation pathway for the desired cell cycle specific hematopoietic cell type followed by the selection of at least one growth factor or cytokine to promote the specific differentiation pathway or to produce a plurality of cells, having the desired cell cycle specific hematopoietic cell type, from the synchronous purified bone marrow stem cells when the synchronous purified bone marrow stem cells are contacted with the growth factor or cytokine at the predetermined phase of the cell cycle. There is no disclosure in Hagihara of the selection of these factors with respect to the "predetermined phase of the cell cycle" with respect to "favoring a specific differentiation pathway" or generating a "plurality of cells having the desired cell cycle specific hematopoietic cell type" when the synchronous purified bone marrow stem cells are contacted with the growth factor or cytokine at the predetermined phase of the cell cycle.

Again, the cells in Hagihara were randomly subcultured "every week" with no disclosure regarding the claimed method of the subculturing with regard to phase of the cell cycle.

Claims 2-4, 7-9, 12-13, and 29-31 are directly or indirectly dependent on claim 1 as an underlying claim, and the arguments and limitations of claim 1 apply to claims 2-4, 7-9, 12-13, and 29-31 as well.

Applicants respectfully submit that remaining claims 1-4, 7-9, 12-13, and 29-31 fulfill the requirements of 35 U.S.C. §102(b), thereby placing these claims in condition for allowance, and request the Examiner's reconsideration accordingly.

IX. The Rejection of Claims 1-13 and 29-31 under 35 U.S.C. §103(a) over Hagihara Taken with Yan, Klabusay, Ramsfjell, and Messner is Traversed, but Rendered Moot in Part

The Examiner has rejected claims 1-13 and 29-31 under 35 U.S.C. 103(a) as unpatentable over Hagihara et al. (J. Immunol. Methods 253: 45-55 [2001]) in view of Yan et al. (Blood, 96(11; part 1): 680a (November 2000) ("Yan")); Klabusay et al. (Blood 100(11): 4118 (November 2002) ("Klabusay")); Ramsfjell et al. (Blood 88(12): 4481-4492 (December 1996) ("Ramsfjell")); and Messner et al. (Blood 70(5): 1425-1432 (November 1987) ("Messner")). Applicants traverse the rejection and respectfully request reconsideration of these claims.

The Patent Office alleges in pertinent part:

> The reference by Hagihara et al. is relied upon as explained above for the disclosure of a method for the production of differentiated hematopoietic cells from bone marrow hematopoietic stem cells by changing the cytokine cocktail combination of steel factor (SCF), thrombopoietin (TPO) and FLT-3 ligand (FLT3) to other factors including GM-CSF.
>
> The cited reference by Hagihara et al. does not explicitly recite that the culturing of cells in the presence of steel factor (SCF), thrombopoietin (TPO) and FLT-3 ligand (FLT3) promotes synchronous progression of the cells through the cell cycle. However, the reference by Yan et al. clearly teaches that the combination of factors SCF, TPO and FLT-3 in the culture medium stimulates the hematopoietic bone marrow cells to enter into synchronous cell cycle from resting state....
>
> The cited reference by Hagihara et al. clearly teaches incorporation of factor GM-CSF in the culture medium for differentiation of hematopoietic bone marrow stem cells but the reference is lacking particular disclosure about the use of G-CSF. However, the reference by Klabusay et al. teaches that hematopoietic stem cells are able to regenerate hematopoiesis in all lineages and that addition of G-SSF in particular will significantly increase the number of maturated cells including granulocytes (see abstract). The reference by Ramsfjell et al. teaches that the use of factor SCF enhances megakaryocyte differentiation and production from stem cells.
>
> Therefore, it would have been obvious to one having ordinary skill in the art at the time the claimed invention was made to modify method of Hagihara et al. by adding G-CSF and steel factor (SCF) during subsequent culturing/subculturing steps

with a reasonable expectation of success in producing differentiated hematopoietic cells including megakaryocytes and granulocytes because the prior art teaches and suggests the use of G-CSF and SCF for enhancing production of granulocytes and megakaryocytes. It is well known that platelets are products of megakaryocytes. Thus, the claimed invention as a whole was clearly *prima facie* obvious, especially in the absence of evidence to the contrary.

Further, the reference by Messner et al. teaches that cell cycle studies and stem cell engraftment studies indicate that the higher than normal proportions of multipotential hematopoietic cells are present in S phase during progression of the hematopoietic cells through the cell cycles (see abstract). Thus, one of skill in the art would have been motivated to contact the hematopoietic stem cells with maturation factors at the time of cell progression through S-phase for the expected benefits in maximizing yields of matured differentiated hematopoietic cells derived from the stem cells. Thus, the claimed invention as a whole was clearly *prima facie* obvious, especially in the absence of evidence to the contrary.

The claimed subject matter fails to patentably distinguish over the state art as represented be the cited references. Therefore, the claims are properly rejected under 35 USC § 103. [Pp. 6-8.]

Applicants respectfully disagree, in part for reasons already discussed above with respect to Hagihara and in Dr. Quesenberry's Declaration, namely, Hagihara also does not disclose or suggest contacting synchronously cycling cells with a growth factor or cytokine "at a predetermined phase of the cell cycle." Rather, Hagihara discloses a vague, imprecise, and random period of time ("every week" – without even any discussion of the day, time, or duration of cell cycle), unlike the Applicants, whose claims are directed to a specific phase of the cell cycle ("predetermined phase of the cycle"), regardless of when in time that phase occurs. In essence, Applicants are claiming a method based on cell cycle, while Hagihara discloses a method based on time.

Yan, Klabusay, Ramsfjell, and Messner, taken either alone or in combination together, fail to supply the deficiencies of Hagihara.

With respect to Yan, the Yan culture was quiescent (97.4% G_0/G_1 phase; 1.5% S phase), but was stimulated by cytokines to enter into cycle as early as 24 hours to yield a fast-dividing population and a slow-dividing population, whereas the cells of the present invention

are cultured from dormancy to synchronous cycles and then stimulated by exposure "at a predetermined phase of the cell cycle," namely, a two-step process – first synchronization, then differentiation into the desired cell cycle specific type, as also noted in Dr. Quesenberry's Declaration.

With respect to Klabusay and Ramsfjell, Applicants respectfully submit that while these references may disclose the generation of various hematopoietic lineages, neither of these references, either alone or in combination with each other or with Hagihara and/or Yan, discloses or suggests generation of at least one specific hematopoietic cell type from a synchronous population of stem cells by exposure to at least one growth factor or cytokine "at a predetermined phase of the cell cycle," as also discussed in Dr. Quesenberry's Declaration.

With respect to Messner, this work is irrelevant to the present invention, as it found variations in frequencies of clonogenic precursors in the normal donor population, but also included marrow from leukemic patients, which cannot be equated with normal marrow. The cell cycle was addressed primarily to determine the proportion of clonogenic precursors in S-phase by preincubation with tritiated thymidine, rather than synchronizing the cell cycles or by exposure of a synchronous population of stem cells "at a predetermined phase of the cell cycle." Messner fails to describe or suggest the claimed method of selecting a phase in the cell cycle to yield cell cycle specific cells, as discussed in Dr. Quesenberry's Declaration.

Nor would one of ordinary skill in the art be motivated to combine Hagihara, Yan, Klabusay, Ramsfjell, and Messner to arrive at the present invention. The present invention is not a combination, simple substitution, or improvement of known elements or methods to yield a predictable result. One of ordinary skill in the art would not have considered it "obvious to try" with any reasonable expectation of success.

None of these references describes or suggests the selection of "a predetermined phase of the cell cycle" or differentiation "hotspot" as a method for selecting a specific differentiation pathway to yield cell cycle specific differentiated hematopoietic cells. None of

these references describes or suggests the reversibility of the "predetermined phase of the cell cycle" or differentiation "hotspot" (see Example 1 and Figures 3-5). A mere reference to S-phase for initiation of unspecified cell differentiation (e.g., Messner) would not describe or suggest the present invention.

Thus, the unpredictability of the present invention goes far beyond a combination, simple substitution, or improvement of known elements or methods and would not have been "obvious to try."

As discussed in Dr. Quesenberry's Declaration, in the present application, the data showed that Lineage^{negative}Rhodamine^{low}Hoescht^{low} ("LRH") cells synchronized and then sub-cultured separately inductive differentiation cocktail (GM-CSF, G-CSF, and steel factor) prior to cell division showed marked variations in differentiated cell production with the first cell cycle transit (Example 1; Figures 1-4). **Surprisingly, megakaryocyte differentiation and proliferative granulocyte differentiation were amplified at G_0 to mid-S phase, whereas non-proliferative granulocyte differentiation was amplified at G_0 to late S phase (Example 1; Figures 1-6).**

According to Dr. Quesenberry's Declaration, **these results are surprising, because the differences provide evidence for a flexible system for hematopoietic regulation in which multiple different outcomes can occur dependent on cell cycle phase and specific microenvironmental influences as part of a continuum of reversible phenotypic shifts (in contrast to a hierarchical model) with continuous change in a reversible fashion.** None of the references cited by the Patent Office addresses this concept or teaches or suggests this concept.

Applicants note that claim 1 is an underlying claim for claims 2-13 and 29-31 and that the arguments that apply to claim 1 also apply to these claims.

Applicants respectfully submit that claims 1-13 and 29-31 fulfill the requirements of 35 U.S.C. §103(a), thereby placing these claims in condition for allowance, and request the Examiner's reconsideration accordingly.

CONCLUSION

It is believed that all outstanding rejections have been addressed by this submission and that all the claims are in condition for allowance. If discussion of any amendment or remark made herein would advance this important case to allowance, the Examiner is invited to call the undersigned as soon as convenient.

In view of the foregoing amendments and remarks, the present application is respectfully considered in condition for allowance. An early reconsideration and notice of allowance are earnestly solicited.

Applicants hereby request a two-month extension of time for the Amendment and accompanying materials. If, however, a petition for an additional extension of time is required, then the Examiner is requested to treat this as a conditional petition for an additional extension of time and the Commissioner is hereby authorized to charge our deposit account no. 04-1105 for the appropriate fee. Although it is not believed that any additional fee (in addition to the fee concurrently submitted) is required to consider this submission, the Commissioner is hereby authorized to charge our deposit account no. 04-1105 should any fee be deemed necessary.

Respectfully submitted,

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